

## Statistical Analysis Plan

**RICHMOND**  
Pharmacology

**SPONSOR'S REFERENCE NUMBER: MMV\_ P218\_15\_01**

**EUDRACT NUMBER: 2016-001933-29**

**RPL STUDY CODE: C16009**

### STATISTICAL ANALYSIS PLAN

**A Phase I study to investigate the safety, tolerability and pharmacokinetic profile and food effect of P218 in healthy adult volunteers**

**FINAL VERSION 1.0, 03 APR 2017**

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## LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation or Specialist Term	Explanation
$\lambda_z$	terminal elimination rate constant
AE	adverse event
Ae <sub>t1-t2</sub>	amount of unchanged drug and metabolite excreted into the urine within time spans
Ae <sub>48h</sub>	amount of unchanged drug and metabolite excreted into the urine from time 0 to 48 hours
Ae%	Percent of dose recovered unchanged in the urine
AUC <sub>∞</sub>	area under the plasma concentration versus time curve from time 0 extrapolated to infinity
AUC <sub>last</sub>	area under the plasma concentration versus time curve from time 0 to the last quantifiable concentration
BLQ	below the limit of quantitation
CL/F	total apparent oral clearance
CL <sub>r</sub>	renal clearance of unchanged drug and metabolite
C <sub>max</sub>	maximum observed plasma concentration
CSR	clinical study report
CI	confidence interval
CV	coefficient of variation
CYP	Cytochrome P450
DBP	diastolic blood pressure
ECG	electrocardiogram
FDA	Food and Drug Administration
HR	heart rate
ICF	informed consent form
IMP	Investigational Medicinal Product
LLQ	lower limit of quantitation
MedDRA	medical dictionary for regulatory activities
PD	pharmacodynamic(s)
PDF	portable document format
PI	principal investigator
PK	pharmacokinetic(s)
PT	preferred term
QTcB	QT interval corrected for heart rate using Bazett's formula
QTcF	QT interval corrected for heart rate using Fridericia's formula
RBC	red blood cell
RPL	Richmond Pharmacology Ltd
SAD	single ascending dose
SAE	serious adverse event
SAP	statistical analysis plan
SAS	statistical analysis software
SD	standard deviation
SOC	system organ class
SBP	systolic blood pressure
TEAE	treatment-emergent adverse event
TESAE	treatment-emergent serious adverse event
TFL	tables figures and listings
t <sub>1/2</sub>	terminal elimination half-life
t <sub>max</sub>	time to maximum observed plasma concentration

## Statistical Analysis Plan



$V_z/F$	apparent volume of distribution
WHO	world health organization
WNCBP	women of non-childbearing potential

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## 1. INTRODUCTION

The purpose of this Statistical Analysis Plan (SAP) is to define the variables and analysis methodology to address the study objectives.

Protocol Version 2 dated 22 Aug 2016 was used in the preparation of this SAP.

Pharmacokinetic parameters will be calculated by MMV or its designee. Richmond Pharmacology Ltd (RPL) is responsible for performing statistical analyses. Tables, figures, and listings will be produced using Statistical Analysis Software (SAS) V9.3 or higher.

## 2. STUDY OBJECTIVES AND ENDPOINTS

### 2.1 Study objectives

The objectives of this study are:

#### Primary

- To investigate the safety and tolerability of single escalating oral doses of P218 when administered to healthy volunteers (men and women of non-childbearing potential [WNCBP]) under fasted conditions.

#### Secondary

- To describe the pharmacokinetics (PK) of P218 and its major glucuronide metabolite (P218 acyl glucuronide) in healthy volunteers (men and WNCBP) after administration of single escalating oral doses
- To investigate the effect of a high fat meal on the PK and safety/tolerability of P218.

#### Exploratory

- To perform ex vivo bioassay analysis against malaria parasites to confirm that P218 acyl glucuronide is active against *Plasmodium* parasite and determine if other active metabolites are formed.
- To assess the effects of P218 on serum folate levels in healthy subjects
- To evaluate the cardiovascular safety profile of P218, assessing qualitative and quantitative electrocardiogram (ECG) variations from baseline following dosing, in particular any effects on the QTc interval.
- To identify inherited genetic factors which may (1) predict response to treatment with P218, (2) explain variability in drug PK/pharmacodynamics (PD), (3) predict susceptibility to drug-drug interactions, or (4) predict the occurrence of safety issues. The aim of such exploratory research will be to develop a better understanding of intrinsic and extrinsic factors that may affect the PK of P218 in human subjects.
- Exploration of Cytochrome P450 (CYP) and Uridine diphosphate glucuronosyltransferase (UGT) isoforms. Exploration of other enzymes and transporters in accordance with evolving data.

## 2.2 ENDPOINTS

### Primary

- Safety and tolerability of P218: Incidence, severity and relationship to the investigational product of observed and self-reported adverse events (AEs) during 11 days post administration of a single oral dose of P218 to healthy volunteers.

### Secondary

- Estimation of the following PK parameters over 11 days post administration of a single dose of P218 (in fasted and fed cohorts) using non-compartmental methods:  $AUC_{last}$ ,  $AUC_{inf}$ ,  $C_{max}$ ,  $T_{max}$ ,  $t_{1/2}$ ,  $CL/F$  (for parent only) and  $V_z/F$  (for parent only).

### Exploratory

- To determine the efficacy of P218 against parasites using an ex-vivo malaria assay:  $IC_{50}$
- Absolute change from baseline P218 on serum folate levels over time

## 3. STUDY DESIGN

### 3.1 Overall study design

#### Part A

This is a randomized, double-blind, placebo-controlled, parallel group, Phase I study which will assess the safety, tolerability, PK of single (SAD) ascending oral doses of P218 in healthy subjects (men and WNCBP).

#### Part B

This is an open-label, randomised fed/fasted crossover study in healthy subjects (men and WNCBP) to examine the food effect of a high fat meal on the PK, safety and tolerability of P218.

This study incorporates the use of an adaptive design.

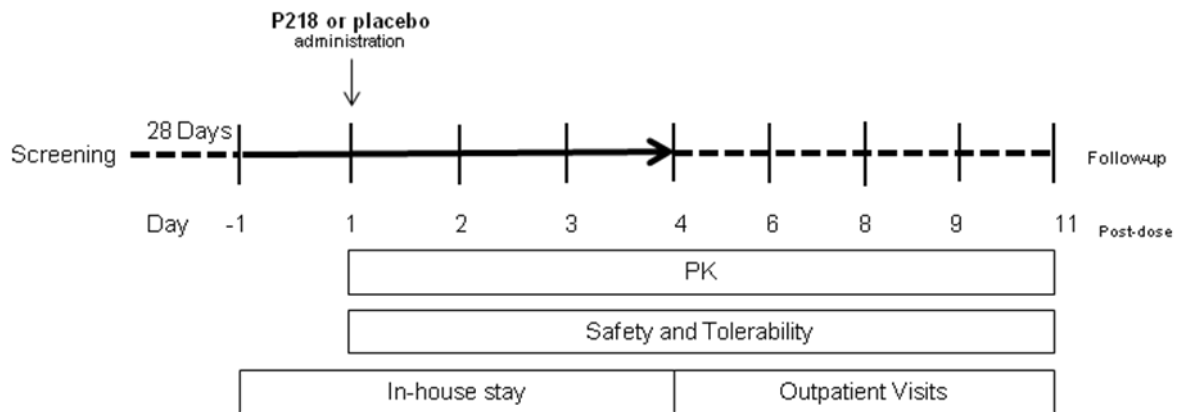
#### **Part A (SAD)**

In Part A, the safety, tolerability and PK of single ascending doses of P218 will be studied in healthy men and WNCBP. Part A is a double-blind randomised, placebo-controlled, parallel group, ascending dose study and will comprise up to eight fasted cohorts (8 volunteers in each) that will receive a SAD of P218. Within each cohort 6 subjects will receive a single dose of P218 and 2 subjects will receive a single dose of matching placebo (3:1 ratio).

Subjects will be screened within 28 days prior to entering the study on Day -1. Each subject will receive verbal and written information followed by signing of the Informed Consent Form (ICF) prior to any screening procedures taking place. Subjects will be admitted to the study unit on Day-1 and will be discharged by Day 4. All subjects will attend the unit for an outpatient visit on Days 6, 8, 9 and a

follow-up visit 11 days post-dose. All the assessments performed during the study are detailed in the study schedule of assessments. Study design features may be adapted according to the Adaptive Features.

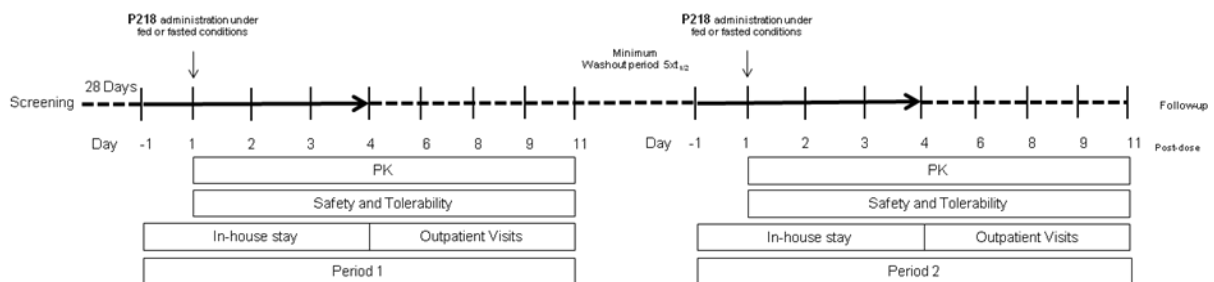
## Part A flow chart



## Part B (Food effect)

For the pilot food effect evaluation, a separate cohort of 8 subjects will receive a P218 dose associated with the targeted efficacious exposure (in human subjects in fasted conditions) in an open-label fashion at two occasions separated by a minimal washout of  $5 \times T_{1/2}$  (that will not exceed 3 weeks). The treatment conditions (fed-fasted) will be randomized according to a two-way crossover design. For the fed period, a standard high fat breakfast is given as per Food and Drug Administration (FDA) guidance; the breakfast is given 30 minutes prior to the scheduled dosing time, and should be completed 10 minutes before dosing.

## Part B flow chart



## 3.2 Randomization and Blinding

Subjects in this study will be assigned to a treatment regimen according to a randomization schedule generated by a statistician using PROC Plan.

Subjects who meet the eligibility criteria will be randomly assigned on Day 1. In Part A subjects will be randomly assigned in a 3:1 ratio to either P218 or placebo. In Part B, all subjects will receive P218 but will be randomly assigned to the two treatment sequences (fed followed by fasted or fasted followed by fed).

## 4. STATISTICAL ANALYSES

All data will be listed and separate listings will be produced for Parts A and B.

In general, descriptive statistics for continuous variables will include number of non-missing values, arithmetic mean, standard deviation (SD), median, minimum, and maximum, by treatment group.

Descriptive statistics for PK parameters will include number of non-missing observations, arithmetic mean, SD, arithmetic coefficient of variation (CV), 95% confidence interval (CI) of the arithmetic mean, geometric mean, geometric CV, median, minimum and maximum, by treatment group.

Categorical variables will be summarized using frequency counts and percentages by treatment group.

Treatment group refers to pooled placebo and dose cohorts of P218 for Part A and to fasted vs. fed for Part B. Parts A and B will be analysed separately.

Baseline will be defined as the last value of the assessment recorded prior to first administration of Investigational Medicinal Product (IMP). For post-dose assessments the first scheduled value will be used for summary analysis if repeated measurements are made at a time-point.

A subject who withdraws prior to the last planned observation in a study period will be included in the analyses up to the time of discontinuation.

All subjects that were enrolled and received a dose of the study drug will be included in the data analysis. Subjects will be analysed as treated (i.e. in accordance with the study drug received, even if different from the one to which they were randomised).

Deviations from the planned analyses will be described in the final integrated clinical study report.

### 4.1. Analysis Populations

The **safety population** will consist of all subjects who received their dose of study drug. Subjects in this population will be used for the safety analyses.

The **PK population** will consist of all subjects in the safety population who have at least one concentration measurement, even if Below the Limit of Quantitation (BLQ). The PK population will be used for PK summaries.

The **PD population** will consist of all subjects in the safety population who have sufficient serum folate levels + red blood cell (RBC) folate levels/Ex-vivo malaria assay data. The PD population will be used for PD summaries.

Inclusion and exclusion from each analysis set will be decided and documented at the Data Review Meeting prior to database lock.

## **4.2. Sample Size and Power**

Because the nature of this study is primarily exploratory, the p-values from hypothesis tests and the CIs obtained in the statistical analyses are not intended to make inferences but to identify the potential patterns and quantify the uncertainty of estimation. The following factors were taken into consideration when determining the sample size:

### **Part A (SAD):**

Because the primary objective is an initial assessment of safety, each treatment group is limited to 6 subjects receiving P218. Administration of P218 to 6 subjects in each dose group provides a 47%, 62%, 74%, or 82% probability of observing at least 1 occurrence of any AE with a true incidence rate for a given dose group of 10%, 15%, 20%, or 25%, respectively.

Furthermore, it is assumed that pooling the data for the 2 placebo subjects who received placebo from each cohort will provide an adequately sized control group.

### **Part B (Food effect):**

This is a pilot evaluation designed to determine whether P218 PK is impacted by food. Historically 8 participants has proven sufficient to characterize the preliminary effects of food on safety and PK of a new chemical entity in healthy subjects. Therefore, the size of the current cohort (n=8) is considered adequate at this stage of the drug development process.

## **4.3. Demographics, Baseline Characteristics, and Subject Disposition**

All subjects will be included in the summary of subject disposition, which will summarize the frequency and percentage of subjects screened, treated, who completed or discontinued the study, along with reason for discontinuation, and the number of subjects in the analysis populations, by pooled placebo and dose groups for Part A and overall for Part B. Demographics and baseline characteristics will be summarized for all subjects by the same groups as for disposition on the safety population. If the remaining populations are different from the safety population, separate demographic tables may be produced.

## **4.4. Safety Analysis**

Safety analyses will be performed on the safety population, and will be reported by treatment group if not specified otherwise. Safety analyses will include an analysis of all AEs, ECGs, clinical laboratory data, and vital sign measurements and will be presented using descriptive statistics. No inferential statistical analyses are planned on the safety parameters of this study.

### **Adverse Events**

The incidence of treatment-emergent (after dosing) AEs (TEAEs) will be summarized using the safety population. The Medical Dictionary for Regulatory Activities (MedDRA) dictionary Version 19.0 (or higher) will be used to classify all AEs reported during the study by System Organ Class (SOC) and Preferred Term (PT). A summary of AEs including the incidence of subjects who experience TEAEs and incidence of TEAEs (number of events) will be presented by pooled placebo, pooled active and dose groups for Part A, and by fed/fasted state for Part B. The

summary will include all TEAEs, TEAEs by maximum severity, TEAEs with suspected relationship to study drug, treatment-emergent serious AEs (TESAEs), TESAEs by maximum severity and TESAEs with suspected relationship to study drug. In addition, TEAEs and TESAEs will be summarized by SOC and PT, by pooled placebo, pooled active and dose group for Part A, and by fed/fasted state for Part B. Tables by SOC and PT will be presented for all TE(S)AEs, TE(S)AEs by maximum severity and T(S)AEs with suspected relationship to study drug. In study Part A, AEs will be categorized by the treatment being received (i.e., P218 at actual dose or placebo). In study Part B, AEs will be categorized by the actual state (i.e., fed or fasted). Serious AEs (SAEs) and AEs resulting in withdrawal from the study will be listed. Subjects having multiple AEs within a category (e.g., overall, SOC and PT) will be counted once in that category. For maximum severity tables, a subject's most severe event within a category will be counted. In each table, SOC and PT within SOC will be presented in descending order of overall incidence rate (alphabetical order will be used in case of equal rates).

## Medical history

Medical history data will be coded using the MedDRA dictionary Version 19.0 (or higher) and listed.

## Previous and concomitant medications

All previous (taken and stopped before dosing) and concomitant (taken after dosing even, if started predose) medications will be coded using the World Health Organization (WHO) Drug Dictionary version June 1, 2016 and will be listed per treatment.

## Study drug administration

Study drug administration data will be listed.

## Vital signs

Vital signs data (systolic and diastolic blood pressure [SBP and DBP], heart rate [HR], temperature) will be listed. Out-of-reference-range values (SBP 80-140mmHg, DBP 40-90mmHg, HR 40-100bpm) and postural drops (supine - standing) (SBP>20mmHg, DBP>10mmHg) will be flagged. Summary statistics of absolute values and changes from baseline will be calculated for each parameter by each treatment group.

## Laboratory data

Safety Laboratory parameters (blood chemistry hematology, coagulation) will be listed and values outside the laboratory reference ranges will be flagged as high (H) or low (L). Absolute values and changes from baseline (continuous variables) will be summarized by each treatment group by scheduled time. The first value

will be used for summary analysis if repeated measurements are made at the same scheduled time.

For summary statistics, the lab value with "<" will be replaced with numeric value by removing the "<" sign. In the listings the values will be displayed as originally reported by the laboratory.

## ECG

All ECG data automatically measured by ECG devices (PR, QRS, QT, QTcB, QTcF and HR) and overall ECG evaluation will be listed. The ECG data, along with changes from baseline will be summarised using descriptive statistics. For summary statistics the average of the ECG readings at the time-points will be calculated.

Furthermore, categorical analysis of QTcF data will be presented for each individual ECG as follows:

- Maximum absolute QTcF interval prolongation
  - QTcF interval < 450ms
  - QTcF interval  $\geq$  450 ms
  - QTcF interval  $\geq$  480 ms
  - QTcF interval  $\geq$  500 ms
- Maximum increase from baseline in QTcF interval
  - QTcF interval increases from baseline < 30ms
  - QTcF interval increases from baseline  $\geq$  30 ms
  - QTcF interval increases from baseline  $\geq$  60 ms

## Physical examination

Physical examination data will be listed.

## 4.5 Pharmacokinetic Analysis

All plasma (and if applicable urine) concentration data of P28 and metabolite P28 acyl glucuronide will be listed and summarized at each time point by treatment group using descriptive statistics. The figures of individual and overlaid individual concentrations vs. actual time, and of mean ( $\pm$  SD) and median ( $\pm$  range) concentrations versus nominal time on linear and semi-log scale will be plotted. If nominal time is used in an analysis, concentrations with a sampling time deviation >10% of the nominal time will be excluded from the analysis.

For all tabulation, graphical representation and calculation purposes, when a BLQ value occurs in a profile before the first measurable concentration, it is assigned a value of zero concentration. When a BLQ value occurs after a measurable concentration in a profile and is followed by a value above the Lower Level of Quantitation (LLQ), then the BLQ will be omitted following visual inspection of the serum concentration versus time profile to assess the appropriateness of this assignment. When a BLQ value occurs at the end of a collection profile (after the last quantifiable concentration), the value is treated as missing data. When two BLQ values occur in succession, the profile is deemed to have terminated at the

first BLQ value and any subsequent concentrations are omitted from PK calculations.

Samples with no reportable value due to a bioanalytical issue or missing samples should be set to missing, and will not be included in the PK calculations.

The individual plasma concentration data for P218 treated subjects, with actual sampling dates and times calculated relative to the dose, will be used to derive the PK parameters by non-compartmental analyses.

The following PK parameters will be derived for P28 and metabolite P28 acyl glucuronide, using standard methods:

Plasma:

- area under the plasma concentration versus time curve from time zero to the last quantifiable concentration ( $AUC_{last}$ )
- area under the curve from time zero extrapolated to infinity ( $AUC_{\infty}$ , calculated as  $AUC_{last} + C_{last} / \lambda_z$  where  $C_{last}$  is the last quantifiable concentration)
- maximum observed plasma concentration ( $C_{max}$ )
- time to first maximum observed plasma concentration ( $T_{max}$ )
- terminal elimination half-life ( $T_{1/2}$ )
- terminal elimination rate constant ( $\lambda_z$ )
- total apparent clearance (CL/F) for parent only
- apparent volume of distribution ( $V_z/F$ ) for parent only

Urine (if applicable):

- amount of unchanged drug and metabolite excreted into the urine within time spans ( $Ae_{t1-t2}$ , ...)
- amount of unchanged drug and metabolite excreted into the urine from time zero to 48 hours ( $Ae_{48h}$ )
- percent of dose recovered unchanged in the urine ( $Ae\%$ )
- renal clearance of unchanged drug and metabolite (CL<sub>r</sub>)

All PK parameters will be listed and summarized by treatment group using descriptive statistics.

Some PK parameters may not be calculated for all or some subjects if the concentration data are not deemed to be amenable to evaluation. Explanations for PK parameters that could not be estimated will be provided in the clinical study report (CSR).

To preliminarily assess dose proportionality for  $C_{max}$  and AUC in Part A, dose-normalized plasma  $C_{max}$ ,  $AUC_{last}$  and  $AUC_{\infty}$  will be calculated and summarized descriptively. In addition a power model with log(PK parameter) as response variable and log(dose) as predictor will be fitted to the data for plasma  $C_{max}$ ,  $AUC_{last}$  and  $AUC_{\infty}$ . A slope of 1 in this model corresponds to dose proportionality. The slope

will be estimated with its 2-sided 90 % and 95 % CI. In addition to visual inspection techniques, a lack-of-fit test (using a model with dose as categorical variable added to the above specified model) will be used to determine the validity of the power model.

A food effect will be assessed in Part B using a mixed effect model with log(PK parameter) as the response variable. Treatment sequence, period and food status (fasted vs. fed) will be included in the model as fixed effects, and subject as a random effect. This model will be applied to the parameters  $C_{max}$ ,  $AUC_{last}$  and  $AUC_{\infty}$ . Based on the model, the estimated food effect (fed – fasted) and its 90 % CI will be presented. Values will be exponentiated to provide the geometric means for each group ( $\mu_{fed}$  and  $\mu_{fasted}$ ), the ratio of the geometric means ( $\mu_{fed}/\mu_{fasted}$ ) and the ratio's 90% CI. The absence of a food effect will be concluded if the 90 % CI of the ratio  $\mu_{test}/\mu_{reference}$  is fully contained within the acceptance range (0.8, 1.25) for  $C_{max}$  and AUC parameters.

#### **4.6. Pharmacodynamic Analyses**

Pharmacodynamic data (serum folate levels and ex-vivo malaria assay) will be listed and absolute (observed values) as well as changes from baseline will be summarized at each time point by treatment group using descriptive statistics. For serum folate levels the figures of mean ( $\pm$  SD) changes from baseline for each treatment group will be plotted against time.

Bioassay analysis individual subject results will be plotted over time.

ECG analysis may be performed and will comprise of adjudicated (manually over-read by a cardiologist) ECG triplicates selected from each timepoint. The analysis will be compliant with the correct recording and manual adjudication of ECGs in thorough QT/QTc studies. QTcF will be used for ECG analyses, unless there is a substantial heart rate effect, in which case the correction used for QTc (the most appropriate heart rate correction) will be calculated under blinded conditions by the statisticians. Details of the analysis, if deemed necessary, will be given in a Supplementary SAP.

#### **4.8 Methods for Withdrawals, Missing Data, and Outliers**

The individual plasma concentration data, and the actual time for IMP administration and blood sampling will be used throughout the analyses. If there is any doubt in the actual time a sample is taken, then the scheduled time will be used. For PK data analysis, please see Section 4.5 for the handling of missing and BLQ values. No further imputation of missing values will be performed for the analysis of dose proportionality or food effect. For PD data analysis, there will be no imputation for missing values.

If medication dates are incomplete and it is not clear whether the medication was concomitant, it will be assumed to be concomitant.

AEs with unknown start date/time will be assumed to be treatment-emergent unless the end date/time is known to be before the first administration of IMP. Otherwise missing or partial dates will be listed as such.

There will be no further imputation of missing data i.e. subjects who prematurely discontinue from the study will not be included in summary statistics or analyses beyond the time of discontinuation. If a subject is withdrawn from study treatment but continues to participate in the study, the data collected will be included according to the treatment initially received/randomized for the safety set.

Depending on the extent of missing values, the appropriateness of the methods described for handling missing data may be reassessed prior to database lock (to examine the sensitivity of results to handling of missing data).

## **4.9 Protocol deviations**

A protocol deviation is defined as a lack of compliance with the protocol which may interfere with the outcome of the trial. The protocol deviations will be identified and reviewed throughout the study, reviewed by principal investigator (PI)/Sponsor and graded as minor or major. The final review of protocol deviations, including any impact on populations and analyses, will be performed at the Blind Data Review Meeting prior to database lock. The protocol deviations will be listed.

## **5. CONVENTIONS**

In Part A, listings will be ordered by cohort, treatment and subject number; in Part B, listings will be ordered by subject number. All listings will include all enrolled subjects.

All listings with post-baseline data will have study day (defined as date minus date of dosing plus one day) presented for Part A and study period and day presented for Part B.

For all tables except PK parameter tables, descriptive statistics for min, max will be presented with the same decimal digits as the original values, and with one more decimal place than the original data for mean, standard deviation and median. Percentages will be presented with one decimal digit if they are larger than zero and smaller than 100%. If the count is zero, percentages will not be presented. If the percentage is 100%, no decimal digit will be presented.

For PK, the individual and summaries of PK parameter results will be reported with 3 significant digits in the listings and in the tables with the following exceptions:

- $T_{max}$  will be presented as follows: individual minimum, and maximum values will be reported exactly as the raw data are reported
- % CV will be presented to 1 decimal place.

Page layout of tables and listings in landscape mode will be produced as Portable Document Format (PDF). Details of page layout will be provided in the tables figures and listings (TFL) shell document.